Predator odours as reproductive inhibitors for Norway rats

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Abstract. We examined the influence of predator odour on reproductive output of Norway rats (*Rattus norvegicus*). Naïve laboratory rats responded to predator chemical cues with reduced litter size and skewed sex ratio. We found that exposure to predator urine had its greatest effect on implantation and maintenance of implantation when predator urine was applied to the bedding of rats during the first third of gestation. Based on the physical appearance of corpora lutea and uterine implantation scars, we found that the reduction in litter size was due to resorption of the embryos during the early part of gestation. Subsequently, we discovered that the reduction in litter sizes in rats exposed to predator urine could be attributed to suppressed progesterone levels affecting implantation of embryos. Chronically high corticosterone levels did not suppress reproductive output. Suppression of reproduction also occurred when rats were exposed to urine of conspecifics housed under high population densities. The evolutionary adaptive response for reduced litter size is to produce high-quality offspring in an environment where food resources are scarce. The fact that rats respond to certain chemical signals in predator urine in a similar fashion may be fortuitous, and may have more to do with the coincidence that the urine contains similar cues resulting from protein digestion in carnivores and protein catabolism in nutritionally deprived rodents, rather than specific predator–prey adaptations.

Introduction

Reproductive traits in rodents are affected by a number of environmental, social and chemosensory factors, e.g. the nutritional status of females will influence ovulation rate and litter size (Hamilton and Bronson 1985), as will exposure of females to other rodents of various social status (Steiner et al. 1983; Huck et al. 1988). Other well-described influences include synchronisation of ovulation amongst female cohorts (Whitten 1956), acceleration or delay of puberty (Vandenbergh 1969; Lombardi and Vandenberg 1977), pregnancy block owing to stress, and failure to implant blastocysts when female rodents are exposed to the odour or urine of strange males (Bruce 1959). Frequently the magnitude of these effects is species- and strain-specific (MacNiven et al. 1992).

The majority of these studies on reproductive inhibition have focused on intraspecific influences of semiochemicals and how they influence reproductive output and behaviour in females. A few studies have focused on between-strain influences or interspecific influence, although the source odour generally is still confined to rodents.

During our investigations on the effects of predator odour on rodent reproduction and repellency, we found that female rats exposed to cat urine during pregnancy had reduced litter sizes at parturition (Voznessenskaya 2002). Exposure to predator odour also caused disruptions of the oestrous cycle (Voznessenskaya et al. 1992). These effects bear striking similarities to the studies of the effects of rodent urine odour on intraspecific rodent reproduction. If such similarities are broadly based, then similarities in mechanisms of perception, reproductive physiology, and chemical nature of the stimulus might be anticipated.

This study was conducted to address some of these issues of similarity. Specifically, we set out to determine if a sensitive period existed for the effect and, if possible, to narrow the timing of the critical period when the stimulus exerts its reproductive effect.

Materials and methods

Test subjects

We used 3–4 month old Norway rats (Rattus norvegicus) from an outbred laboratory population as a model system. Before the start of the experiments, females were housed in groups of 3–4, and males were housed singly. Experimental rooms were illuminated on 14:10 hours light:dark schedule, and maintained at 20°C. Food and tap water were provided *ad libitum*. Virgin females in pro-oestrus/oestrus, as determined by vaginal cytology, were chosen for the mating experiments. Sexually experienced males that were not mated in the 14 days before the test were used as sires. The morning after pairing, the females were checked for successful mating, as indicated by the presence of a vaginal plug. Successfully mated females were then housed singly.

Reproductive output

For each experimental group, the total number of offspring per female was counted and the sex ratio determined. In addition, we also measured the gestation period.

Determination of implantation sites in utero and post-implantation loss

Female were necropsied 2–3 days after giving birth and the presence and number of implantation scars in their uteri were recorded. Each scar indicates the site of placental/foetal attachment during pregnancy. Scars are roughly 2 mm in diameter and can be counted easily under a microscope (Bacon and McClintock 1994). The numbers of pups carried in utero were compared to the numbers of pups found at birth and thus the amount of post-implantation pup loss could be quantified. The number of implantation scars was compared with number of corpora lutea to detect pre-implantation loss.

Collection of urine

Urine from domestic cats (*Felis catus*) was used as a source of predator chemical cues. These cats normally hunt for mice and have mice as part of their diet. If needed, additional meat was added to their diet. Freshly voided urine was frozen (–22°C). Once defrosted, urine was used only once. Non-predator urine was obtained from guinea pigs and Norway rats. For collection of the urine from Norway rats held in overcrowded conditions, 10–12 animals were placed in a standard size cage for 14 days. Animals received a reduced per capita ration so as to maintain 80% of normal body mass and water ad libitum. Individuals of these species (as appropriate for the type of treatment specified in the experiment) were placed into metabolic stainless-steel cages overnight, and urine was collected and stored using the method described above. Urine was collected and stored at –22°C.

'Open field' test with added stress

An 'open arena' (1.5 × 1.5 m) with bright lights was used. Pregnant females were placed for 15 minutes in the centre of the arena on the 1st, 4th and 7th day of gestation. During the test, we also used a buzzer, which made a loud noise, every 5 minutes. In addition, rats were handled roughly to physically induce stress. Blood samples were drawn after each test for progesterone and corticosterone assay.

Assay for progesterone and corticosterone

Rats within each treatment were randomly assigned to one of four cohorts. Blood samples (300–400 µL) were obtained from the rear foot pad (Miller et al. 1997) every 3rd day for each cohort for each of the treatments for the first 7 days of gestation. This minimises the handling and sampling of individual rats, while allowing a detailed study of changes in hormonal pattern as a function of time and treatment. Our experience shows that this method of repeated blood sampling has no long-term effect on visible scarring associated with traditional tail sampling technologies (Miller et al. 1997). Samples were centrifuged and the plasma frozen at –20°C until subsequent analysis. Plasma progesterone and corticosterone were assayed (in triplicate) by the coat-a-tube radio immuno assay (RIA) method (Diagnostic Products, Los Angeles, California, ≈50 µL/assay; Miller et al. 1997).

Experimental design and protocol

The experimental method consisted of applying 1 mL of a test solution to the bedding of pregnant rats every other day for different time durations. This application maximised the likelihood of physical and odour exposure of the test stimulus to the female. In the first experiment, six treatment levels were used:

1. tap water (WAT), as a negative control;
2. urine from guinea pigs maintained on a vegetarian diet (vegetables, grains and water ad libitum), as a urine control (GPU);
3. urine from domestic cats maintained on a feral mouse diet (CU), as a model stimulus representing unadulterated predator urine. Cats were maintained on the feral mouse diet for 14 days before urine collection;
4. urine from the same feral cats as CU treated with 4% $HgSO_4$ to yield urine where small peptides, amines, and sulfur compounds were precipitated (CU-$HgSO_4$). The supernatant was used as the test stimulus and did not contain indicators of a carnivorous diet (Nolte et al. 1994);
5. urine from cats on a vegetarian diet (CU-v). After collecting urine from the cats used in the above treatments, we switched the animals to a vegetarian diet (oatmeal, peaches, milk and bread ad libitum) for 14 days before urine collection; and
6. urine from overcrowded rats (OWU), as source of conspecific chemical signals of high population density (intrinsic factor for regulation of population density).

After mating, females were randomly assigned to treatment groups: WAT (n = 20), GPU (n = 20), CU (n = 20), CU-$HgSO_4$ (n = 21), CU-v (n = 26) and OWU (n = 21).

Mean differences among treatment groups were determined in separate analyses for the number of pups and sex ratios using a fixed-effects analysis of variance. Post-hoc differences among treatment groups were determined using a Spjotovoll-Stoline test for unequal sample sizes (Statistica; StatSoft Inc, Tulsa, Oklahoma).

In the second set of experiments, we determined the period during gestation when rats were sensitive to predator urine. The first three treatments focused on exposing pregnant rats to cat urine for varying lengths of time while the blastula was in the oviduct.

1. In the first treatment, cat urine (1 mL) was added to the bedding of female rats (n = 22) immediately after mating and left in place for 24 h (E24). During this period of development, the single fertilised cell is found in the oviduct. After 24 h of exposure to the cat urine the treated bedding was replaced with clean bedding.
2. In the second treatment, 1 mL of cat urine was added to the bedding of females (n = 20) immediately after mating and left in place for 36 h (E36). During this period, the single cell cleaves into two cells, while still remaining in the oviduct. Following the 36 h exposure period, the bedding was changed as above.
3. In the third treatment, the procedures were repeated as per above (n = 21), but the exposure period was 48 h (E48). During this period, the blastula progresses to the four-cell stage within the oviduct.
4. The fourth treatment consisted of applying 1 mL of cat urine to the bedding of female rats (n = 20) that had been mated 48 h before application of urine, and the exposure continued for the next 48 h (E48D). During this stage of development, the early blastocyst migrates to the uterus but remains free from attachment to the uterus. Implantation of the blastocyst occurs 6–8 days after fertilisation.
5. A fifth treatment was applied at 12 days post-fertilisation (embryo is well-formed and attached in the uterus) in which cat urine (1 mL) was applied to bedding on 12th day and left in place for 48 h (MGP) (n = 20). After this exposure, bedding was replaced with clean material.
6. The sixth treatment (WAT) served as the negative control (n = 21). Water (1 mL) was applied to the bedding every other day throughout gestation.
7. The seventh treatment served as positive control (n = 20). Cat urine (CU) (1 mL) was applied to bedding every other day throughout gestation. This treatment has been shown to result in 40% reduction in litter sizes relative to water control in previous studies (Voznessenskaya 2002).

The data were statistically analysed as for the previous experiments.

Results and discussion

The number of pups born to female rats varied as a function of treatment (F = 28.61, df = 4,102, P < 0.001). Post-hoc analysis indicated that diet, specifically urine products derived from meat diets, was important in producing the reproductive inhibitory effect. Females exposed to urine derived from cats maintained on a mouse diet had the smallest litter sizes (Table 1). All other treatments resulted in litter sizes similar to those seen for females exposed to water (i.e. negative control), except for the group treated with urine from overcrowded rats. For this treatment, litter size was significantly reduced relative to both of the controls. Sex ratios also were affected by the treatment (F = 16.53, df = 4,102, P < 0.001). Fewer female pups were born to rats exposed to urine derived from cats maintained on mouse diets and to rats exposed to urine from overcrowded conspecifics. All other treatments resulted in sex ratios similar to the negative control, which itself was similar to 0.5.

Table 1. The influence of exposure to predator odours and conspecific odours on alternate days during gestation on litter size and sex ratio in Norway rats.

Type of treatment	Litter size	Sex ratio (females/total number of pups)
Exposure to cat urine (CU)	4.8 ± 0.4	0.31 ± 0.07
Exposure to CU treated with $HgSO_4$ to remove indicators of carnivorous diet (CU-$HgSO_4$)	9.3 ± 0.4	0.50 ± 0.11
Exposure to urine from cats on vegetarian diet (CU-v)	8.3 ± 0.4	0.50 ± 0.09
Exposure to tap water (WAT)	8.6 ± 0.2	0.51 ± 0.11
Exposure to guinea pig urine (GPU)	8.6 ± 0.2	0.52 ± 0.12
Exposure to urine of overcrowded conspecifics	6.7 ± 1.2	0.41 ± 0.07

When urine was deficient in compounds characteristic of meat digestion, it had no effect on rat reproduction, irrespective of species, or how the deficiency of these products was obtained, i.e. via diet restriction or chemical removal. Urine derived from rats housed in crowded conditions suppressed the reproductive output of rats at rates comparable to rats that were exposed to predator urine. These data suggest that catabolism of muscle mass due to starvation results in urine that contains reproductively inhibitory materials

Table 2. Effect of timing of predator odour exposure during gestation on litter size in Norway rats.

Type of treatment	Litter size	Sex ratio (females/total number of pups)
Exposure to cat urine (CU) every other day during gestation	6.0 ± 1.3	0.36 ± 0.03
Exposure for 24 h after mating (E24)	8.4 ± 0.5	0.45 ± 0.07
Exposure for 36 h after mating (E36)	6.5 ± 0.3	0.41 ± 0.05
Exposure for 48 h after mating (E48)	6.4 ± 0.3	0.41 ± 0.04
Exposure for 48 h, 2 days after mating (E48D)	6.9 ± 0.3	0.39 ± 0.09
Exposure for 48 h on day 12 (mid-gestation) (MGP)	10.7 ± 0.3	0.49 ± 0.03
Control—exposure to tap water (WAT)	10.7 ± 0.3	0.50 ± 0.10

In the second experiment, litter size varied among treatments ($F = 37.65$, df = 6,137, $P < 0.001$). Litter size was largest for the negative control (WAT = 10.7 ± 0.3), and smallest for the positive control (CU = 5.9 ± 1.3), representing a 44.9% decrease in litter size. Once the embryo was implanted into the uterus, a 48 h exposure (MGP) to cat urine had no effect on litter size relative to the control (Table 2). Exposure to cat urine while the blastula was in the oviduct resulted in decreased litter sizes relative to the control, with the larger reductions resulting from 48 h exposure. Litter reductions were achieved at the expense of female pups ($F = 5.37$, df = 6,137, $P < 0.001$). The proportion of females born in the negative control group (WAT) was 0.506 ± 0.011. The proportion of females born in the positive control treatment (CU) was 0.361 ± 0.022: a 28.7% decrease in the expected proportion of females. The change in sex ratio across treatments followed that for overall litter size (Table 2).

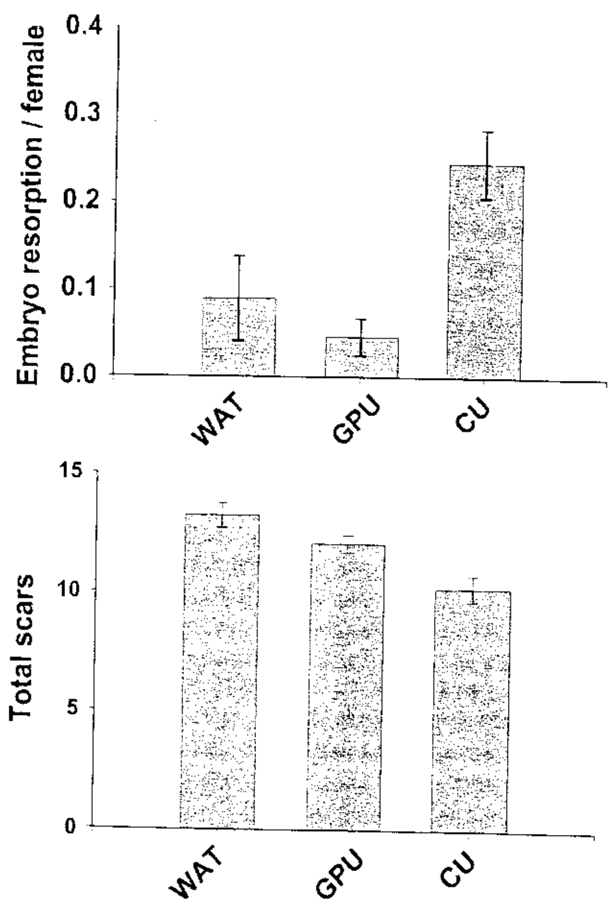

Figure 1. The influence of exposure to urine odours from cats (CU), guinea pigs (GPU) and tap water control (WAT) on (upper) embryo resorption and (lower) implantation in Norway rats. There were no significant differences between the water and guinea pig urine groups.

Although the precise, critically sensitive period during gestation has not been established, we do know that it occurs within the first half of gestation, and that an acute exposure to predator urine of 48 h up to 3 days after mating is sufficient to produce the negative effect on reproduction.

The observed changes to litter size, sex ratio, and gestation period brought about by early exposure to predator urine may involve one or more of the following mechanisms: (1) decreased ovulation; (2) delayed, reduced and/or differential fertilisation of the egg by sperm; (3) reduced implantation; (4) differential resorption of fertilised eggs or blastocoels; and (5) delayed implantation coupled with sex-linked differential survival of the blastocoel.

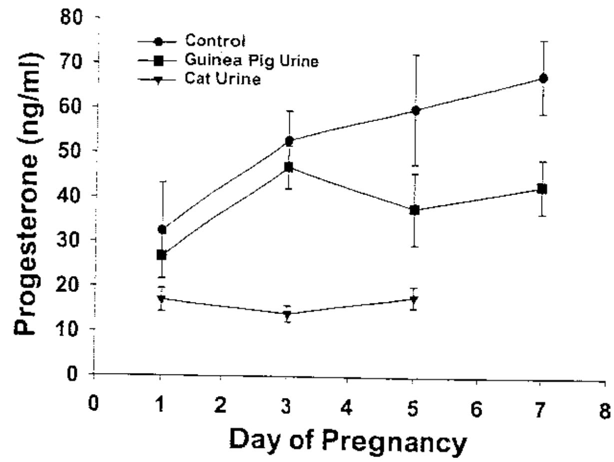

Figure 2. Plasma progesterone levels (mean ± sem) in Norway rats exposed to different urine odours (tap water control, guinea pig urine or cat urine) every other day during gestation.

Progesterone is a key ovarian hormone produced by the corpora lutea, and is responsible for the maintenance of the fertilised egg, preparation of the endometrium, and maintenance of pregnancy. Factors that disrupt progesterone production could lead to the altered reproductive success observed for rats exposed to predator urine. In follow-up experiments, we monitored plasma progesterone and corticosterone (a hormone released in response to stress) in female Norway rats during early gestation. In addition to groups exposed to urine odours, and control treatment, there was another group in which rats were handled roughly to physically induce stress ('open field' test with added stress). As we observed in our previous studies, female rats exposed to cat urine had smaller litter sizes. Based on the number of corpora lutea and implantation scars, it appeared that reduction in litter size was due to reduced implantation and resorption of embryos during the early part of gestation (Figure 1). Both of the control groups had similar embryo resorption rates, while the cat urine group experienced a higher embryo resorption rate per female (Figure 1a). These effects were also observed between treatments for the number of implantation scars (Figure 1b). Consistent with the morphological evidence was the observation that plasma progesterone levels were dramatically suppressed in rats exposed to cat urine relative to levels observed in the water control group and for rats exposed to guinea pig urine (Figure 2). We did not observe statistically significant differences in plasma corticosterone levels for rats exposed to predator and non-predator urine, while rough handling of animals caused a clear elevation of corticosterone (Figure 3). Plasma corticosterone levels were unrelated to the two measures of reproductive output, i.e. number of live births and resorption of embryos, with stress per se not influencing reproductive output (Figure 4).

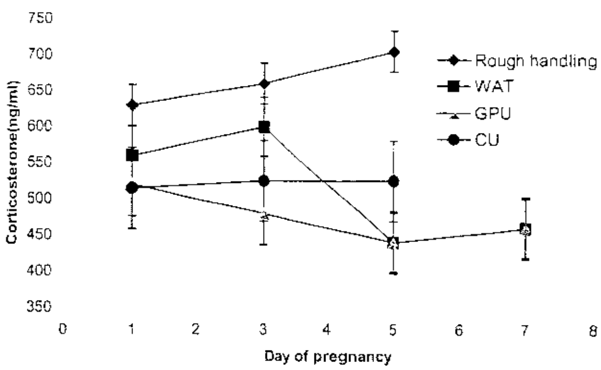

Figure 3. Plasma corticosterone levels (mean ± sem) in Norway rats exposed to urine odours (cat urine, CU; guinea pig urine, GPU; or control tap water, WAT) every other day during gestation or to rough handling in an 'open field' test with added stress.

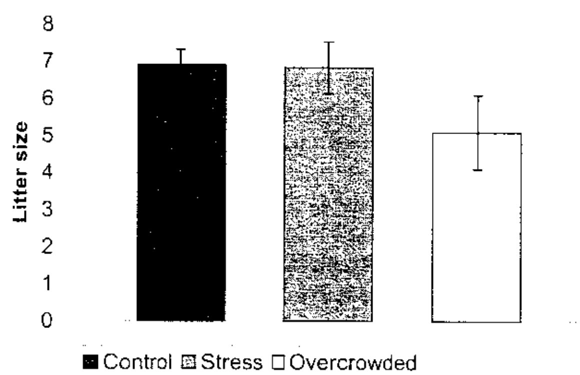

Figure 4. The influence of stress or exposure to urine derived from overcrowded conspecifics on litter size in Norway rats where: control = exposure to tap water every other day during gestation; stress = 'open field' test with added stress; overcrowded = exposure to urine from overcrowded conspecifics every other day during gestation.

Conclusion

We do not believe that reduction in litter size is attributable to an adaptive response by rodents to predator odours. Rather, we propose the following interpretation. Urine contains information about the identity of individuals, reproductive status, and dominance status. We postulate that urine also contains information about environmental quality as reflected by nutritional status. Investigation of urine from a variety of sources would serve as an efficient way to integrate environmental information. During times of food depletion, an individual could assess the nutritional status of the population. If food becomes limiting, rodents will begin to catabolise their own muscle protein and the urine will contain larger amounts of protein degradation products. These signals could serve to trigger mechanisms that would affect reproduction. Given that the generation time of rodents is short, complete reproductive inhibition may not be adaptive. However, reduced reproduction may be beneficial. Reduced reproduction would relieve energetic constraints on lactating females that might otherwise jeopardize survival if a full litter size were attempted.

Litters are biased toward producing males when predator or rat catabolic urine is used as a stimulus. This is consistent with theory on reproductive value. Even with reduced litter size, females may still experience lower survival probabilities during reproduction and lactation in food limiting environments because of energetic constraints. However, males would be less constrained by such energetic considerations. Thus, their survivorship probabilities may be higher than females, and by implication their value in contributing to fitness would also be higher. So then, why should rodents reduce reproduction when presented with predator urine? Predators on rodent diets would produce urine with many of the same rodent-derived metabolic products. It is only coincident that the two urines produce the same effect: an artifact of the experiments.

Rodents cause considerable economic damage to field and fruit crops on an annual basis. The magnitude of the problem is exemplified for a single commodity, apples. In the United States of America (USA) alone, rodents cause US$90 million of damage each year. Efforts to control rodents responsible for apple crop damage in the USA include the application of 321,000 pounds of herbicides for habitat management and 1000 pounds of acute rodenticides, including strychnine, zinc phosphide and chlorophacinone (National Agricultural Statistical Service, 1996). The most extensive damage occurs at the apex of rodent population cycles. It is our goal to develop a product that will dampen the amplitude of these rodent population cycles, thus resulting in less rodent pressure on crops and, by implication, result in less economic damage. We envision encapsulating active ingredients from urine and auto-catabolism of rodent muscle protein into bait that a rodent will take back to its burrow. Once in the burrow, female rodents would be exposed to the active ingredients on a schedule that would result in reduced reproductive output. This method utilises naturally derived compounds that pose no environmental hazard. Thus, this method should prove useful in reducing our reliance on pesticides with less favorable environmental properties while achieving the goal of reducing rodent populations.

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